Randomized Pharmacokinetic and Pharmacodynamic Comparison of Fluoroquinolones for Tuberculous Meningitis[▽][†][‡]

Guy E. Thwaites,[1,2*] Sujata M. Bhavnani,[3] Tran Thi Hong Chau,[4] Jeffrey P. Hammel,[3]
M. Estée Török,[5] Scott A. Van Wart,[3] Pham Phuong Mai,[4] Daniel K. Reynolds,[3]
Maxine Caws,[2] Nguyen Thi Dung,[4] Tran Tinh Hien,[4] Robert Kulawy,[3]
Jeremy Farrar,[2] and Paul G. Ambrose[3]

*Centre for Molecular Microbiology and Infection, Imperial College, South Kensington, London, United Kingdom[1];
Oxford University Clinical Research Unit, Wellcome Trust Major Overseas Programme, Hospital for Tropical Diseases,
190 Ben Ham Tu, District 5, Ho Chi Minh City, Vietnam[2]; Institute for Clinical Pharmacodynamics,
43 British American Blvd., Latham, New York[3]; the Hospital for Tropical Diseases, Ho Chi Minh City,
Vietnam[4]; and Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom[5]*

Received 17 January 2011/Returned for modification 28 February 2011/Accepted 9 April 2011

Tuberculous meningitis (TBM) is the most lethal form of tuberculosis, and new treatments that improve outcomes are required. We randomly assigned adults with TBM to treatment with standard antituberculosis treatment alone or in combination with ciprofloxacin (750 mg/12 h), levofloxacin (500 mg/12 h), or gatifloxacin (400 mg/24 h) for the first 60 days of therapy. Fluoroquinolone concentrations were measured with plasma and cerebrospinal fluid (CSF) specimens taken at predetermined, randomly assigned times throughout treatment. We aimed to describe the pharmacokinetics of each fluoroquinolone during TBM treatment and evaluate the relationship between drug exposure and clinical response over 270 days of therapy (Controlled Trials number ISRCTN07062956). Sixty-one patients with TBM were randomly assigned to treatment with no fluoroquinolone ($n = 15$), ciprofloxacin ($n = 16$), levofloxacin ($n = 15$), or gatifloxacin ($n = 15$). Cerebrospinal fluid penetration, measured by the ratio of the plasma area under the concentration-time curve from 0 to 24 h (AUC_{0-24}) to the cerebrospinal fluid AUC_{0-24}, was greater for levofloxacin (median, 0.74; range, 0.58 to 1.03) than for gatifloxacin (median, 0.48; range, 0.47 to 0.50) or ciprofloxacin (median, 0.26; range, 0.11 to 0.77). Univariable and multivariable analyses of fluoroquinolone exposure against a range of different treatment responses revealed worse outcomes among patients with lower and higher plasma and CSF exposures than for patients with intermediate exposures (a U-shaped exposure-response). TBM patients most likely to benefit from fluoroquinolone therapy were identified, along with exposure-response relationships associated with improved outcomes. Fluoroquinolones add antituberculosis activity to the standard treatment regimen, but to improve outcomes of TBM, they must be started early, before the onset of coma.

Tuberculous meningitis (TBM) causes death or severe disability in half of those afflicted, yet drug treatment has not changed since the introduction of rifampin in the 1970s (35). While early antituberculosis chemotherapy in combination with adjunctive corticosteroids improves outcomes (33), further improvements are urgently required. The *in vitro* activity of fluoroquinolones against *Mycobacterium tuberculosis* has been recognized for decades (36). While fluoroquinolones are considered essential for the treatment of multidrug-resistant tuberculosis (10) and may have a role in the treatment of drug-sensitive pulmonary tuberculosis (11), their role in the treatment of TBM is uncertain.

Fluoroquinolones may improve TBM outcomes by enhancing the initial antimycobacterial activity of the standard antituberculosis drug regimen. Additionally, they may be useful for patients unable to take isoniazid or rifampin because of patient intolerance or bacterial drug resistance to these agents. The optimal fluoroquinolone agent and exposure for TBM treatment have not been defined. There have been case reports or small case series describing ciprofloxacin, levofloxacin, and moxifloxacin pharmacokinetics (PKs) in TBM treatment (1, 2, 4, 7, 13, 38), but there are no clinical data evaluating gatifloxacin or fluoroquinolone exposure-response relationships for this patient population.

Our aim was to evaluate the pharmacokinetics of fluoroquinolones and exposure-response relationships for the efficacy of these agents in patients with TBM in the context of standard combination antituberculosis chemotherapy. This is the first study comparing the plasma and cerebrospinal fluid (CSF) pharmacokinetics of ciprofloxacin, gatifloxacin, and levofloxacin in patients with TBM and the first to evaluate the contribution of fluoroquinolone exposure to outcomes in this severely ill population.

---

* Corresponding author. Mailing address: Centre for Molecular Microbiology and Infection, Imperial College, Exhibition Road, London, United Kingdom. Phone: 020 75943094. Fax: 020 75943095. E-mail: guy.thwaites@btinternet.com.

MATERIALS AND METHODS

Setting and study participants. Study participants were recruited from the Hospital for Tropical Diseases (HTD) in Ho Chi Minh City, Vietnam, a 500-bed secondary and tertiary referral hospital for infectious diseases. All adults (>14 years of age) with suspected TBM were eligible to enter the study. Patients were

ineligible if they were pregnant, fluoroquinolones were contraindicated for any reason, or consent was not obtained.

The ethical and scientific committees of the HTD and the Health Service of Ho Chi Minh City and the Oxford Tropical Research Ethics Committee approved the study protocol. Written informed consent for study participation was obtained from all patients or their relatives. The International Standard Randomized Controlled Trial number is ISRCTN07062956.

Treatment. Patients received daily oral treatment with isoniazid (5 mg/kg of body weight/day; maximum, 300 mg) and rifampin (10 mg/kg/day; maximum, 600 mg) for 9 months. Oral pyrazinamide (30 mg/kg/day; maximum, 2 g/day) and intramuscular streptomycin (20 mg/kg/day; maximum, 1 g/day) were given for the first 3 months. Ethambutol (30 mg/kg/day; maximum, 1.2 g/day) was substituted for streptomycin in HIV-infected adults and was added to the regimen for 3 months for those previously treated for tuberculosis. Adjunctive dexamethasone was given for the first 6 to 8 weeks, as described previously (33). Drugs were given by nasogastric tube for those unable to swallow. No HIV-infected patients received antiretroviral drugs because they were unavailable at the time of the study.

Patients were allocated by a computer-generated sequence of random numbers into blocks of 10 (Microsoft Excel, 1997 to 2003) to receive the above-described treatments, alone or in combination with open-label oral ciprofloxacin (750 mg/12 h), levofloxacin (500 mg/12 h), or gatifloxacin (400 mg/24 h), for the first 60 days.

Laboratory investigations. Paired blood and cerebrospinal fluid (CSF) specimens were taken for the assay of fluoroquinolone concentrations upon admission and at days 2, 7, 30, 60, and 270 of treatment. Unless clinically contraindicated, the timing of the lumbar puncture was randomized to 0 to 8, 8 to 16, or 16 to 24 h following antituberculosis drug administration. Two additional blood specimens were taken within 24 h of drug administration at predetermined, randomly assigned times before and after the lumbar puncture. Thus, for each patient, on each sample collection day, one randomly timed CSF sample and two randomly timed plasma samples were collected; this procedure was repeated on up to five occasions per patient.

High-pressure liquid chromatography tandem mass spectrometry (LC/MS/MS) methods were developed at the Ordway Research Institute and used to determine ciprofloxacin and gatifloxacin concentrations in plasma and CSF and levofloxacin concentrations in CSF. Plasma levofloxacin concentrations were determined by using a high-pressure liquid chromatography mass spectrometry (LC/MS) method, which was also developed at the Ordway Research Institute. Samples (0.050 ml) were deproteinated and analyzed by using a Phenomenex Luna Phenyl-Hexyl column (150 by 4.6 mm, 5 μm) with mobile phases of 0.1% formic acid in water and 0.1% formic acid in acetonitrile interfaced with either an ABSciex API5000 (LC/MS/MS) or an Agilent LC/MSD (LC/MS) instrument.

All *M. tuberculosis* isolates were tested for drug susceptibility by using the 1% proportion method on Lowenstein-Jensen medium. The laboratory performing this test is accredited by the WHO and is a Western Pacific Region WHO supranational tuberculosis reference laboratory. The MIC of ofloxacin for each isolate was determined by the microtiter dilution method. Ofloxacin MIC values were determined and correlated to the potencies of other quinolones by using potency relationships documented in the literature. For example, levofloxacin was more active than ofloxacin, as it is the active component of the racemic mixture that is ofloxacin. The MIC values for the culture-negative patients were designated the MIC_{50} values based on those reported in the literature (27, 28, 29).

Potential fluoroquinolone resistance was investigated with locked nucleic acid probe real-time PCR to detect mutations in *gyrA* (39).

Assessment of clinical outcome. Clinical data were recorded prospectively over 270 days of therapy and entered into an electronic database. Clinical outcomes assessed after 270 days of treatment included survival, death or disability, and disease relapse. Time to death, time to fever resolution, and time to coma clearance were assessed over this period. Coma was assessed by the Glasgow coma score (GCS), which is based on the best eye, verbal, and motor responses (31). The lowest possible score (the sum of the 3 responses) is 3 (which indicates deep coma), while the highest is 15 (fully awake person). Neurological disability was assessed by using Rankin and "simple-questions" scores as previously described (33). Disease relapse was defined as the onset of new focal neurological signs or a decrease in the GCS of 2 points or more for 2 or more days following more than 7 days of clinical stability or improvement at any time after randomization. Time to fever resolution was defined as the time in days from randomization to the observation of a maximum daily body temperature of lower than 37.5°C for more than five consecutive days. Time to coma clearance was defined as the time in days from randomization to the observation of a GCS of 15 for more than two consecutive days.

Determination of plasma and CSF fluoroquinolone exposures. A sequential population pharmacokinetic analysis approach, using nonlinear mixed-effects methodology as implemented in the NONMEM, version 6, software package (6), was used to evaluate the sparsely sampled plasma and CSF concentration-time data for each fluoroquinolone. One- and two-compartment models with first-order absorption and elimination were evaluated to describe the plasma concentration-time data for each drug (2, 15, 24, 25).

Structural model development. Differential equations and initial conditions (ICs) describing the amount of drug in the depot (A_{po}), plasma (A_p), and nonspecific tissue (A_T) compartments are shown in equations 1, 2, and 3, respectively:

$$\frac{dA_{po}}{dt} = -k_a \cdot A_{po}, \text{IC} = F \cdot \text{dose} \tag{1}$$

$$\frac{dA_p}{dt} = k_a \cdot A_{po} + \frac{\text{CL}d}{V_p} \cdot A_T - \left(\frac{\text{CL}}{Vc} + \frac{\text{CL}d}{Vc}\right) \cdot A_p, \text{IC} = 0 \tag{2}$$

$$\frac{dA_T}{dt} = \frac{\text{CL}d}{Vc} \cdot A_p - \frac{\text{CL}d}{Vp} \cdot A_T, \text{IC} = 0 \tag{3}$$

For the one-compartment models evaluated, each drug was parameterized by using a first-order absorption rate constant (k_a), the apparent central volume of distribution (Vc/F) and plasma clearance (CL/F), and an absorption lag time (ALAG). For the two-compartment models evaluated, two additional parameters were included, the distribution clearance (CLd/F) and peripheral volume of distribution (Vp/F). Apparent PK parameters were calculated due to the absence of intravenous data, which are required to determine the bioavailability (F) for each drug.

Individual plasma PK parameter estimates for each drug were utilized with the equations shown above to generate plasma drug concentrations ($C_p = A_p/Vc$) for use as a driving function to characterize the concentrations of each drug in the CSF (C_{CSF}), as shown in Fig. S1 in the supplemental material. An effect-site compartment model was utilized, which assumed that the total amount of drug entering the CSF from plasma was negligible for each drug, as shown in equation 4:

$$\frac{dC_{CSF}}{dt} = k_{app} \cdot C_p - k_{rem} \cdot C_{CSF} \tag{4}$$

where k_{app} and k_{rem} represent the first-order rate constants for the transfer of the drug to and the eventual removal of the drug from the CSF, respectively.
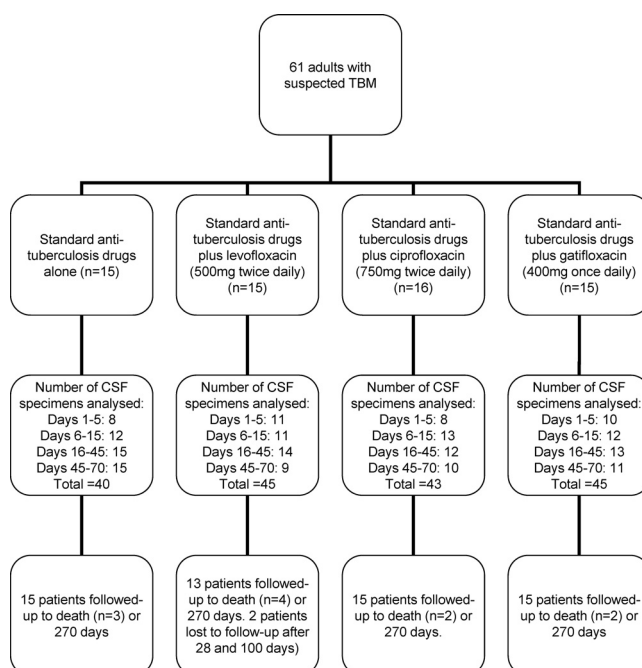

FIG. 1. Flow of participants through the trial.

TABLE 1. Baseline characteristics of all patients recruited for the study

Variable	Value for group receiving:			
	Ciprofloxacin ($n = 16$)	Gatifloxacin ($n = 15$)	Levofloxacin ($n = 15$)	No fluoroquinolone ($n = 15$)
Median age (yr) (min, max)	39 (17, 75)	24 (16, 70)	33 (18, 68)	36 (15, 82)
No. of male patients (%)	10 (62.5)	5 (33.3)	9 (60.0)	12 (80.0)
No. of patients with previous tuberculosis treatment (%)	0	0	2 (13.3)	0
No. of patients with HIV infection (%)	0 (14 tested)	0 (15 tested)	2 (14.3) (14 tested)	1 (13 tested)
Median wt (kg) (min, max)	50 (21, 65)	48 (34, 58)	48 (31, 60)	49 (40, 68)
Median duration of symptoms before treatment (days) (min, max)	14 (6, 60)	16 (6, 40)	16 (4, 33)	13 (6, 36)
Mean GCS (range)	14 (6-15)	13 (3-15)	13 (4-15)	13 (7-15)
No. of patients with cranial nerve palsy present (%)	6 (37.5)	7 (46.7)	5 (33.3)	6 (40.0)
No. of patients with hemiplegia present (%)	2 (12.5)	2 (13.3)	2 (13.3)	1 (6.7)
No. of patients with MRC disease severity grade[a] (%) of:				
I	4 (25.0)	2 (13.3)	3 (20.0)	3 (20.0)
II	9 (56.3)	9 (60.0)	8 (53.3)	9 (60.0)
III	3 (18.8)	4 (26.7)	4 (26.7)	3 (20.0)
Median serum creatinine concn (mg/dl) (min, max)	75 (57, 138)	77 (45, 105)	64 (41, 97)	70 (52, 170)
Median plasma sodium concn (mmol/liter) (min, max)	130 (123, 143)	130 (119, 145)	131 (151, 138)	137 (122, 144)
Median CSF opening pressure (cm H_2O) (min, max)	18 (2, 50)	33 (11, 42)	24 (8, 40)	21 (15, 44)
Median CSF total white cell count (10^3 cells/ml) (min, max)	243 (60, 955)	295 (64, 1,566)	335 (1, 985)	325 (50, 2,200)
Median % of CSF neutrophils (min, max)	39 (2, 91)	14 (1, 75)	22 (2, 74)	50 (4, 90)
Median % of CSF lymphocytes (min, max)	62 (9, 96)	86 (25, 99)	79 (26, 98)	50 (10, 96)
Median CSF total red cell count (10^3 cells/ml) (min, max)	97 (0, 7,200)	37 (0, 80)	60 (0, 2,200)	15 (0, 10,650)
Median CSF total protein concn (mg/dl) (min, max)	145 (27, 312)	91 (40, 368)	105 (24, 606)	99 (38, 485)
Median CSF lactate concn (mmol/liter) (min, max)	4.4 (2.7, 9.2)	6.1 (4.1, 8.9)	4.5 (3.4, 8.2)	5.8 (3.3, 10.7)
Median ratio of CSF/blood glucose (min, max)	0.23 (0.06, 0.38)	0.19 (0.06, 0.38)	0.23 (0.09, 0.41)	0.21 (0.02, 0.62)
No. of patients with acid-fast bacilli seen or cultured from the CSF (%)	11 (68.8)	10 (66.7)	8 (53.3)	10 (66.7)
No. of patients with susceptibility testing result of:				
Fully susceptible	7	5	3	3
Isoniazid resistant	0	1	0	2
Streptomycin resistant	0	2	0	0
Isoniazid and streptomycin resistant	0	0	0	1

[a] According to guidelines from the British Medical Research Council (MRC). Grade I, alert and orientated with no focal neurological signs; grade II, disorientated (GCS of 11 to 14) and/or focal neurological signs; grade III, comatose (GCS of <11) with or without focal neurological signs (9).

The between-patient variability (ω^2) was described for each PK parameter, where possible, by using an exponential error model, assuming that each PK parameter is log-normally distributed. For example, the individual gatifloxacin CL/F equals [population mean gatifloxacin CL/F] $\cdot \exp^{\eta CL/F}$, where $\eta_{CL/F}$ is a Gaussian random error term representing the difference between each of the individual CL/F values and the population mean CL/F, with the distribution of $\eta_{CL/F}$ having a mean of zero and a variance of ω^2. Between-occasion variability was also estimated when necessary. Residual variability was described by using a constant coefficient of variation (CV) error model.

Using the individual *post hoc* parameter estimates obtained for each patient, steady-state plasma and CSF drug exposures, represented by the area under the concentration-time curve over a 24-h period (AUC_{0-24}) for each sampling occasion, were calculated for each individual patient who received a fluoroquinolone. Steady-state AUC_{0-24} values for each patient were averaged across sampling occasions. CSF penetration, as measured by the ratio of the mean steady-state CSF AUC_{0-24} to the mean steady-state plasma AUC_{0-24}, was then calculated.

Univariable analyses. We examined the relationships between plasma and CSF fluoroquinolone exposure and survival, death or disability, and disease relapse by 270 days of treatment and time to death, time to fever resolution, and time to coma clearance. Analyses for time to coma clearance were limited to those patients with a GCS of 14 or lower at the start of treatment. Plasma and

TABLE 2. Summary statistics for selected plasma and CSF fluoroquinolone exposure measures

Drug and dose	Mean AUC_{0-24} (mg · h/liter) (min, max)[a]			Mean CSF AUC_{0-24}/plasma AUC_{0-24} ratio (min, max)[c]
	Plasma	Free-drug plasma[b]	CSF	
Ciprofloxacin				
500 mg twice daily ($n = 2$)	22.5 (14.6, 30.4)	15.7 (10.2, 21.3)	6.97 (4.45, 9.49)	0.31 (0.31, 0.31)
750 mg twice daily ($n = 13$)	31.2 (13.2, 50.0)	21.8 (9.24, 35.0)	6.95[d] (2.30, 14.9)	0.26[d] (0.11, 0.77)
Gatifloxacin at 400 mg once daily ($n = 14$)	41.2 (34.2, 85.7)	32.9 (27.4, 68.5)	20.0 (16.5, 41.5)	0.48 (0.47, 0.50)
Levofloxacin at 500 mg twice daily ($n = 14$)	155 (81.1, 284)	107 (56.0, 196)	94.1 (53.1, 208)	0.74 (0.58, 1.03)

[a] Represents the mean exposure estimates across all occasions for each patient.
[b] Steady-state plasma AUC_{0-24} values for fluoroquinolones were corrected for protein binding based on point estimates for percentages of drug bound to serum proteins of 30%, 20%, and 31% for ciprofloxacin, gatifloxacin, and levofloxacin, respectively.
[c] The ratio of the mean steady-state CSF AUC_{0-24} to the mean steady-state plasma AUC_{0-24}, also referred to as the penetration ratio.
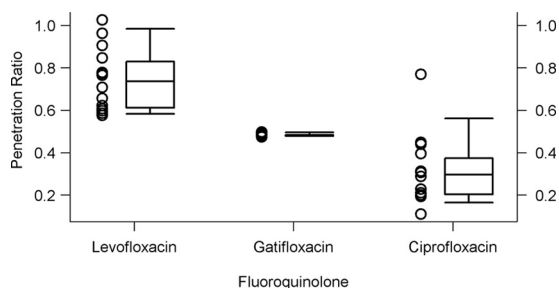[d] Based on data for 12 patents.

FIG. 2. Comparison of CSF penetrations among the fluoroquinolones evaluated. Box plots of the ratio of the mean steady-state CSF AUC_{0-24} to the mean steady-state plasma AUC_{0-24} are shown for each agent. Observed data are represented by open circles.

CSF fluoroquinolone exposure data were each pooled for analysis, and plasma AUC_{0-24} values were corrected for protein binding (5, 8, 23) and normalized by the MIC for *M. tuberculosis* to derive plasma and CSF AUC_{0-24}/MIC ratios, respectively. Univariable analyses were conducted to evaluate both plasma and CSF AUC_{0-24}/MIC ratios as independent exposure variables. Relationships for dichotomous outcomes were examined by using chi-square or Fisher's exact tests for categorical independent variables and logistic regression for continuous independent variables. Relationships for time-to-event outcomes were examined by using log rank tests for categorical variables and Cox regression for continuous variables. Univariable relationships for each outcome were also evaluated for patient age, GCS, creatinine clearance rate, plasma sodium concentration, ratio of CSF/blood glucose, and CSF total protein concentration, lactate concentration, total white cell count, and neutrophil percentage.

Each independent variable was evaluated in its original form and as two- and three-group categorical variables relative to each outcome to account for potential nonlinearity and nonmonotonicity, respectively. Two-group independent variables were constructed by using the resulting split of a classification tree for a given dichotomous outcome and by using a cutoff maximizing the hazard ratio estimate from a univariable Cox regression model for a given time-to-event outcome. Three-group independent variables were constructed by determining a pair of cutoff values that minimized the likelihood ratio P value using either

logistic regression for a dichotomous outcome or Cox regression for a time-to-event outcome.

Multivariable analyses. Multivariable analyses were conducted for each outcome by first constructing a base covariate model using the forward inclusion of independent variables with an entry criterion of a P value of <0.05. Logistic regression was used to evaluate relationships between independent variables and dichotomous outcomes, and Cox regression was used for time-to-event outcomes. Likelihood ratio P values were assessed throughout the forward inclusion process. All non-exposure-independent variables (including the continuous and two- and three-group forms of each variable) were considered for inclusion into the base model. However, once a single form of a given independent variable entered the model, no other forms of the same variable were considered for entry at subsequent steps. The limits placed on the number of independent variables included in a model were based on recommendations by Hosmer and Lemeshow for logistic regression (18). For the time-to-event outcomes, these criteria were similarly followed (since only censored patients limit the available information). Once base models were constructed, exposure-response relationships for each exposure variable were then considered. The resulting covariate-adjusted relationships were assessed to understand the differences relative to those observed with the univariable analyses.

RESULTS

Sixty-one adults with suspected TBM were recruited to the study between April 2003 and January 2005. A TBM diagnosis was confirmed by a positive CSF smear for acid-fast bacilli (31/61 [50.8%]) or mycobacterial culture from 39/61 (63.9%) patients. Fifteen patients were treated with standard antituberculosis drugs alone, and 16 received ciprofloxacin, 15 received levofloxacin, and 15 received gatifloxacin (Fig. 1). Three patients, each receiving one of the fluoroquinolones, died and thus did not contribute any plasma or CSF data. Two patients, both given levofloxacin, were lost to follow-up after 28 and 100 days of treatment, respectively; these patients were included in the time-to-event analyses but excluded from the analyses of dichotomous outcomes. Baseline clinical variables were similar

TABLE 3. Summary of P values and directional assessments for univariable relationships between outcome and independent variables

Independent variable[a]	P value for outcome					
	Survival	Death/disability[c]	Relapse[d]	Time to death	Time to fever resolution[e]	Time to coma clearance[f]
Plasma fluoroquinolone AUC_{0-24}/MIC ratio	0.031 ∩[b]	0.008 U	0.12[b]	0.014 ∩[b]	0.039 U	0.063 U[b]
CSF fluoroquinolone AUC_{0-24}/MIC ratio	0.004 ∩	0.016 U	0.10 ∩	0.003 ∩[b]	0.193	0.454
Age (yr)	0.003(2) \	0.049(2) /	0.041(3) /	0.023(2) \	0.058(2) \	0.353(3)
GCS	0.002(2) /	<0.001(2) \	0.015(3) ∩	<0.001(2) /	0.189(3)	<0.001(3) \
Creatinine clearance (mg/dl)	0.11(2)	0.033(3) U	0.05(3) ∩	0.17(3)	0.013(2) \	0.001(3) U
Plasma sodium concn (mmol/liter)	0.10(3) ∩	0.036(3) ∩	0.13(3)	0.105(3)	0.193(3)	0.053(3) ∩
CSF/blood glucose ratio	0.16(2)	0.21(3)	0.035(2) \	0.054(3) ∩	0.063(3) ∩	0.66(2)
CSF total protein concn (mg/dl)	0.08(3) ∩	0.14(3)	0.12(3)	0.105(2)	0.28(1)	0.299(2)
CSF lactate concn (mmol/liter)	0.032(3) \	0.036(3) ∩	0.008(3) U	0.027(1) \	0.073(3) ∩	0.053(3) U
CSF total white cell count (10^3 cells/ml)	0.006(3) ∩	0.001(3) ∩	0.008(3) ∩	0.006(3) ∩	0.019(3) ∩	0.489(2)
CSF neutrophils (%)	0.028(3) ∩	0.17(3)	0.010(3) U	0.049(3) ∩	0.043(2) \	0.113(2)

[a] For independent-exposure variables, the reported P values and directionalities are those of the three-group variable. Directionality is reported only for P values of ≤0.10. For three-group exposure variables with P values of >0.10, only one case was identified for which the P value was ≤0.10 for another form of exposure. A P value of 0.09 was reported for the relationship between plasma AUC_{0-24}/MIC ratio and relapse. For non-exposure-independent variables, the P value reported is the lowest among the continuous (1), two-group (2), and three-group (3) variables, with the corresponding observed directionality. Directionality is indicated as follows: ∩, the outcome likelihood or time to event tends to be highest for a middle group; U, the outcome likelihood or time to event tends to be lowest for a middle group; /, the outcome likelihood or time to event tends to be highest for higher values of the independent variable; \, the outcome likelihood or time to event tends to be lowest for higher values of the independent variable.

[b] Cases for which the two-group independent exposure variable produced a smaller P value than the P value reported for the three-group form of that particular exposure variable.

[c] Neurological disability was assessed for survivors after 270 days of treatment using the Rankin and "simple-questions" disability scores as previously described (33).

[d] Relapse of disease was defined as the onset of new focal neurological signs or a decrease in the GCS of 2 points or more for 2 or more days following more than 7 days of clinical stability or improvement at any time after randomization.

[e] Time to fever resolution was defined as the time in days from randomization to the observation of a maximum daily body temperature of lower than 37.5°C for more than five consecutive days.

[f] Time to coma clearance was defined as the time in days from randomization to the observation of a GCS of 15 for more than two consecutive days.
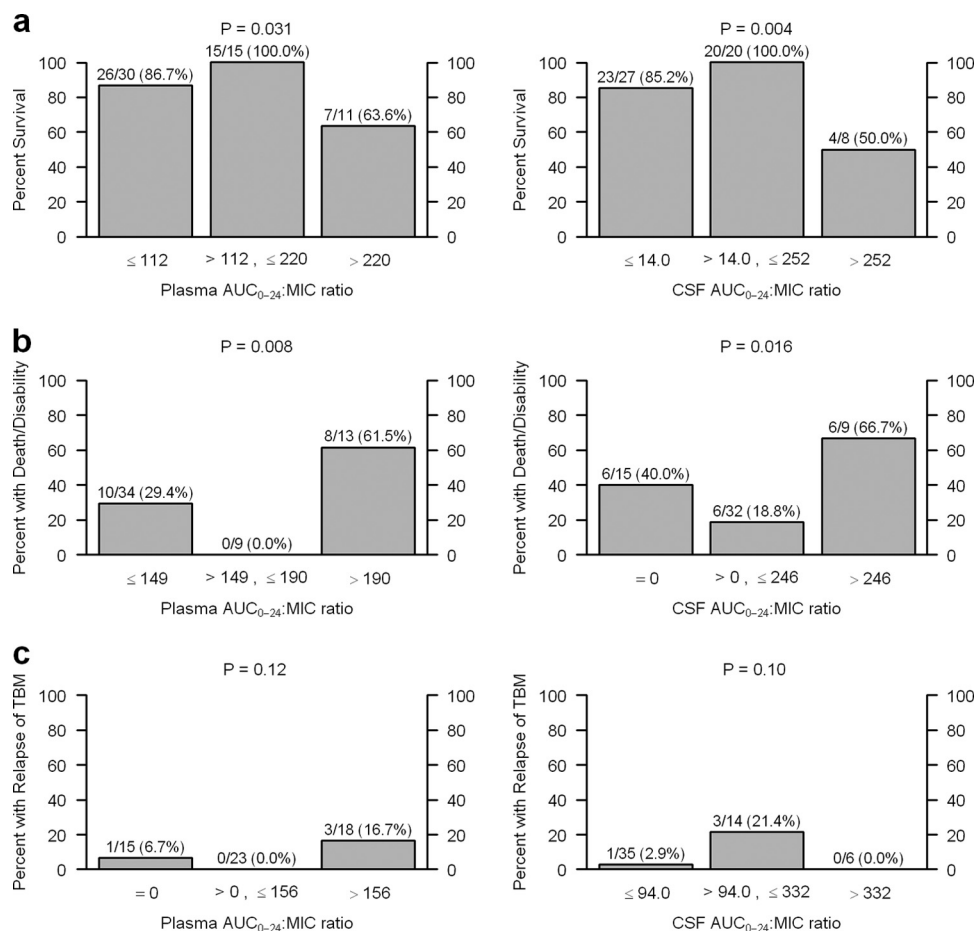
FIG. 3. Univariable relationships between dichotomous and time-to-event outcomes and three-group drug exposure variables.

among the 4 treatment groups (Table 1). Isolates resistant to isoniazid and/or streptomycin were recovered from 6 patients (25% of those tested). None of the isolates were resistant to rifampin or ofloxacin.

Assay performance. Plasma assays were linear over a concentration range from 0.050 to 10.0 μg/ml ($r^2 > 0.984$) for each of the fluoroquinolones. The interday precision (percent CV [%CV]) and accuracy (percent recovery) ranged from 2.19 to 8.01% and 88.7 to 96.0% for ciprofloxacin, 2.30 to 7.08% and 92.0 to 97.7% for gatifloxacin, and 5.20 to 8.21% and 91.0 to 101% for levofloxacin, respectively. CSF assays were linear over concentration ranges from 0.005 to 1.0 μg/ml ($r^2 > 0.982$) for ciprofloxacin and gatifloxacin and from 0.0100 to 5.00 μg/ml ($r^2 > 0.992$) for levofloxacin. The interday precision (%CV) and accuracy (percent recovery) ranged from 2.54 to 8.54% and 90.0 to 113% for ciprofloxacin, 1.91 to 3.55% and 98.3 to 101% for gatifloxacin, and 3.52 to 7.08% and 96.4 to 108% for levofloxacin, respectively.

Evaluation of plasma and CSF fluoroquinolone exposures. As demonstrated by the agreement between population mean predicted steady-state plasma or CSF concentration-time profiles and observed plasma or CSF concentration data shown in Fig. S1 in the supplemental material, the dispositions of gatifloxacin and levofloxacin in plasma and CSF were each best described by a one-compartment model with first-order ab-

sorption and elimination along with an effect-site compartment model. For ciprofloxacin, a two-compartment model with first-order absorption and elimination along with an effect-site compartment model best described plasma and CSF concentration-time data. A summary of the mean parameter estimates and standard errors based on the final population pharmacokinetic model for each fluoroquinolone is presented in Table S1 in the supplemental material. Summary statistics for selected plasma and CSF fluoroquinolone exposure measures are provided in Table 2. As shown in Fig. 2, CSF penetration, measured by the ratio of the mean steady-state CSF AUC_{0-24} to the mean steady-state plasma AUC_{0-24}, was higher for levofloxacin (median, 0.74; range, 0.58 to 1.03) than for gatifloxacin (median, 0.48; range, 0.47 to 0.50) or ciprofloxacin (median, 0.26; range, 0.11 to 0.77) ($P < 0.001$ by a Kruskal-Wallis test). Moreover, penetration ratios for levofloxacin and ciprofloxacin were far more variable than those for gatifloxacin.

Relationship between exposure and outcome. (i) Univariable analyses. A summary of the P values and directional assessments for univariable relationships between outcome and independent variables is shown in Table 3. Univariable relationships between dichotomous and time-to-event outcomes and the three-group fluoroquinolone exposure variables constructed based on these outcomes are shown graphically in Fig. 3. Consistent and statistically significant "U-shaped" rela-
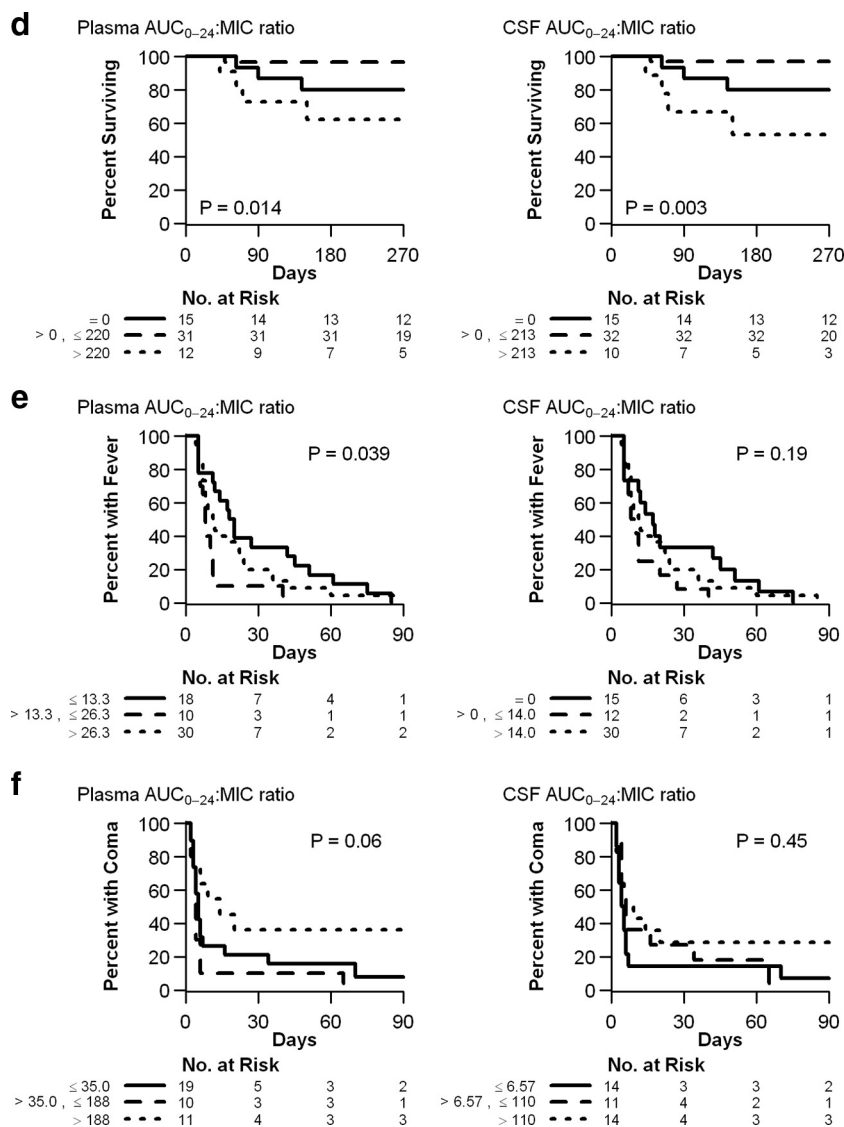
FIG. 3—Continued.

tionships (i.e., apparent nonmonotonic relationship resembling a U or inverted U) were observed between plasma and CSF fluoroquinolone exposures and the following outcomes: survival, death or disability, and time to death. For dichotomous outcomes, higher percentages of less favorable events were observed for patients with both low and high fluoroquinolone exposures than for those with intermediate exposures. For time-to-event outcomes, patients with intermediate exposures had a longer time to death and, with the exception of the relationship based on CSF exposure, a shorter time to fever resolution. Similar U-shaped exposure-response relationships were observed for the remaining outcomes, but statistical significance was not consistently observed for both exposure variables.

The GCS was the baseline independent variable most strongly and consistently associated with poor outcomes among independent nonexposure variables. Low GCS scores were significantly associated with death ($P = 0.002$) and death

or disability ($P < 0.001$) and with longer time to death and time to coma clearance ($P < 0.001$).

(ii) Multivariable analyses. A summary of the P values and directional assessments for relationships between outcome and independent variables included in multivariable base models and individually added three-group exposure variables is shown in Table 4. Multivariable models are not shown for relapse, as there were insufficient numbers of events for assessment. Of the independent variables included in each of the base models, the GCS was most common and, when retained, demonstrated the strongest association with outcome. As shown by the P values and directionality for each exposure variable considered individually for addition to base models, previously observed univariable relationships for three-group exposure variables appeared to be minimally affected by covariate adjustments. The similarity between univariable and multivariable results is most notable for the survival, death-or-

TABLE 4. Summary of *P* values and directional assessments for relationships between outcome and independent variables included in multivariable base models and individually added three-group exposure variables

Variable[a]	*P* value for outcome				
	Survival	Death/disability[d]	Time to death	Time to fever resolution[f]	Time to coma clearance[g]
Independent					
Plasma fluoroquinolone AUC_{0-24}/MIC ratio	0.027 ∩[c]	0.028 U	0.06 ∩[c]	0.052 U	0.78[e]
CSF fluoroquinolone AUC_{0-24}/MIC ratio	0.006 ∩	0.026 U	0.033 ∩[c]	0.22[c]	0.95
Base model independent[b]					
GCS	$0.001_{(2)}$ /	$<0.001_{(2)}$ \	$<0.001_{(1)}$/		$<0.001_{(1)}$ \
CSF total protein concn (mg/dl)		$0.002_{(2)}$ /[e]			
CSF neutrophils (%)			$0.003_{(3)}$ ∩		
Creatinine clearance (mg/dl)				$<0.001_{(3)}$ ∩[e]	
CSF lactate concn (mmol/liter)				$<0.001_{(3)}$ ∩	
CSF/blood glucose ratio				$0.002_{(3)}$ ∩	$0.027_{(3)}$ U[e]

[a] For independent-exposure variables, the reported *P* values and directionalities are those of the three-group variable when added to the base model. Directionality is reported only for *P* values of ≤0.10.

[b] For the independent variables retained in the base model, the *P* value reported is either for the continuous (1), two-group (2), or three-group (3) variable, with the corresponding observed directionality. Directionality is reported for ≤0.10. Directionality is indicated as follows: ∩, the outcome likelihood or time to event tends to be highest for a middle group; U, the outcome likelihood or time to event tends to be lowest for a middle group; /, the outcome likelihood or time to event tends to be highest for higher values of the independent variable; \, the outcome likelihood or time to event tends to be lowest for higher values of the independent variable. As per the Hosmer and Lemeshow guidelines for dichotomous outcomes (18), base multivariable models for survival and death/disability were limited to include 1 and 2 independent variables, respectively. Base multivariable models for time to death, time to fever resolution, and time to coma clearance were similarly limited to 2, 5, and 3 independent variables, respectively. However, as shown by the base models for these outcomes containing 2, 3, and 2 independent variables, respectively, stepwise modeling procedures stopped before these limits were reached.

[c] Cases for which the two-group form of an exposure variable produced a smaller *P* value than the *P* value reported for the three-group form of that variable.

[d] Neurological disability was assessed for survivors after 270 days of treatment using the Rankin and "simple-questions" disability scores as previously described (33).

[e] Notable change in significance (at a *P* value of 0.10) or direction from the univariable *P* value reported in Table 3.

[f] Time to fever resolution was defined as the time in days from randomization to the observation of a maximum daily body temperature of lower than 37.5°C for more than five consecutive days.

[g] Time to coma clearance was defined as the time in days from randomization to the observation of a GCS of 15 for more than two consecutive days.

disability, and time-to-death outcomes, for which U-shaped exposure-response relationships were almost always preserved.

The distribution of baseline independent variables was evaluated across the levels of the three-group exposure variables for plasma and CSF AUC_{0-24}/MIC ratios to probe for the basis for the observed U-shaped exposure-response relationships. Although not always statistically significant (likely a function of the limited sample size), certain differences among those patients with low, intermediate, and high fluoroquinolone exposures were evident. As shown in Table 5, patients with high plasma or CSF exposures were older, had reduced creatinine clearance rates, lower GCSs, higher concentrations of CSF total protein and lactate, and/or lower numbers of CSF white cells and neutrophils than did patients with intermediate exposures. Patients with low exposures were older than those with intermediate exposures but had similar clinical markers of disease severity.

DISCUSSION

The goal of this study was to evaluate the pharmacokinetics of fluoroquinolones and exposure-response relationships for the efficacy of these agents in patients with TBM in the context of standard antituberculosis chemotherapy. As a first step, population pharmacokinetic models describing the disposition of each fluoroquinolone in plasma and CSF were developed. These models were similar to those previously described (24), with the exception of ciprofloxacin, for which the mean population estimates of clearance and volume of distribution were approximately twice those of a previous report (15). Given that the mean population estimate of the terminal half-life was

similar to that of the previous report (15), reduced bioavailability in our patient population may be the likely explanation for this discordance. Ciprofloxacin, unlike gatifloxacin and levofloxacin, is metabolized. Enzyme induction due to coadministration with rifampin may have been a potential factor in this population (3).

CSF penetration was greater for levofloxacin than for gatifloxacin or ciprofloxacin at the doses explored. However, penetration ratios for levofloxacin and ciprofloxacin penetration were highly variable relative to those with gatifloxacin. Since these drugs have similar molecular weights and protein binding (5, 8, 23), CSF penetration differences were likely governed by hydrophobicity/partitioning and the balance between the affinities for influx and efflux active transporters. The higher CSF penetration for levofloxacin was likely related to the unique organic anion transporting polypeptide 1A2-mediated uptake into the brain (20) and an ATP-binding cassette transporter at the blood-CSF barrier (12), which mediates fluoroquinolone efflux out of the CSF (21, 22). Overlapping substrate specificities between the influx and efflux transporters make the vectorial transport of organic anions highly efficient and may best explain differences in CSF penetrations among the three agents. Interestingly, a recent analysis of 4 moxifloxacin-treated adult TBM patients suggested similar CSF penetrations for moxifloxacin and levofloxacin (2). Gatifloxacin's more reliable CSF penetration may be related to the change of its octanol-water partition coefficient as a function of ambient pH (26). When one considers the more predictable CSF penetration and greater *in vitro M. tuberculosis* activity of gatifloxacin than those of levofloxacin (28), drug exposure, as measured by

TABLE 5. Distribution of independent variables in patients with low, intermediate, and high plasma and CSF fluoroquinolone AUC_{0-24}/MIC ratios

Variable[b]	Value for plasma AUC_{0-24}/MIC ratio of[a]:				Value for CSF AUC_{0-24}/MIC ratio of[a]:			
	≤112 (n = 30)	112–220 (n = 15)	>220 (n = 11)	Plasma AUC_{0-24}/MIC ratio P value	≤14 (n = 27)	14–252 (n = 20)	>252 (n = 8)	CSF AUC_{0-24}/MIC ratio P value
Total % of patients (no. of patients with ratio/total no. of patients)	53.6 (30/56)	26.8 (15/56)	19.6 (11/56)		49.1 (27/55)	36.4 (20/55)	14.5 (8/55)	
% death (no. of patients with ratio/total no. of patients)	13.3 (4/30)	0 (0/15)	36.4 (4/11)	0.031	14.8 (4/27)	0 (0/20)	50.0 (4/8)	0.004
% of patients with death/disability (no. of patients with ratio/total no. of patients)	27.6 (8/29)	20.0 (3/15)	54.5 (6/11)	0.16	29.6 (8/27)	20.0 (4/20)	62.5 (5/8)	0.10
% of patients in treatment group (no. of patients with ratio/total no. of patients)								
Ciprofloxacin	50.0 (15/30)	0 (0/15)	0 (0/11)	<0.001	44.4 (12/27)	10.0 (2/20)	0 (0/8)	<0.001
Gatifloxacin	0 (0/30)	66.7 (10/15)	36.4 (4/11)		0 (0/27)	60.0 (12/20)	25.0 (2/8)	
Levofloxacin	0 (0/30)	33.3 (5/15)	63.6 (7/11)		0 (0/27)	30.0 (6/20)	75.0 (6/8)	
No fluoroquinolones	50.0 (15/30)	0 (0/15)	0 (0/11)		55.6 (15/27)	0 (0/20)	0 (0/8)	
Median age (yr) (IQR)	36 (26.5, 47)	23 (19.5, 37)	42 (23.5, 60)	0.07	36 (25.5, 46)	25 (20, 38.5)	47.5 (23, 67.3)	0.10
Median GCS (IQR)	13.5 (12.25, 15)	14 (12, 14)	13 (11, 14.5)	0.79	14 (11.5, 15)	14 (12, 15)	12.5 (11, 14)	0.52
Median creatinine clearance (mg/dl) (IQR)	74.0 (56.6, 104)	87.4 (77.2, 95.8)	64.9 (56.7, 98.4)	0.59	74.1 (57.5, 106)	87.2 (70.2, 96.1)	61.8 (54.5, 90.8)	0.59
Median plasma sodium concn (mmol/liter) (IQR)	133 (129, 137)	129 (124, 131)	131 (128, 135)	0.06	134 (129, 138)	129 (125, 132)	131 (128, 133)	0.044
Median CSF/blood glucose ratio (IQR)	0.229 (0.103, 0.322)	0.213 (0.109, 0.259)	0.260 (0.100, 0.330)	0.84	0.215 (0.108, 0.321)	0.206 (0.101, 0.275)	0.286 (0.145, 0.35)	0.59
Median CSF total protein concn (mg/dl) (IQR)	117 (67.0, 163)	88.0 (59.5, 125)	123 (83.5, 183)	0.36	120 (70, 162)	88.5 (59.8, 127)	114 (75.5, 174)	0.54
Median CSF lactate concn (mmol/liter) (IQR)	5.2 (3.85, 7.45)	5.2 (4.5, 6.25)	5.65 (5.05, 6.2)	0.58	5.2 (3.9, 7.7)	5.3 (4.5, 6.15)	5.6 (5, 6.2)	0.73
Median CSF total white cell count (10^3 cells/ml) (IQR)	284 (234, 474)	300 (275, 345)	240 (104, 550)	0.74	300 (240, 363)	285 (236, 4623)	255 (104, 494)	0.88
Median % of CSF neutrophils (IQR)	45.5 (23.3, 64)	27 (6, 42.5)	10.5 (5, 15.5)	0.009	45 (24.5, 63)	21.5 (8.25, 45.8)	8 (3, 32.5)	0.038

[a] The three-group plasma and CSF AUC_{0-24}/MIC variables were based on the dichotomous survival outcome.
[b] IQR, interquartile range.

the CSF AUC_{0-24}/MIC ratio, is higher and less variable for gatifloxacin, which may translate into improved patient outcomes. Given the lower ciprofloxacin CSF exposure, the lower aforementioned bioavailability in this patient population, the lower *in vitro* activity against *M. tuberculosis* than that of gatifloxacin and levofloxacin (14, 16, 27, 28, 29, 30), and evidence of resistance emergence upon therapy (17), ciprofloxacin should not be the preferred fluoroquinolone for the treatment of TBM.

Using plasma and CSF exposures from the above-described population pharmacokinetic models, subsequent univariable analyses of clinical outcomes revealed intriguing results: consistent U-shaped exposure-response relationships were observed for dichotomous and time-to-event outcomes. For example, significantly higher proportions of death and death or disability were observed for patients with lower or higher plasma and CSF fluoroquinolone exposures than for patients with intermediate exposures. Also, patients with the lowest and highest plasma and CSF fluoroquinolone exposures were significantly more likely to die more rapidly than those with intermediate exposures.

The paradoxical relationship between high CSF exposures and poor outcomes may be explained by a colinear association with increased disease severity. As identified here and previously (19, 33, 34), the baseline GCS was the strongest independent predictor of a poor TBM outcome. In addition, reduced numbers of CSF white cells and increased lactate CSF concentrations have also been strongly linked to poor outcomes (32, 33). In the analyses described here, we found that those patients with the highest CSF fluoroquinolone exposures had lower GCSs, the lowest numbers of CSF white cells, and the highest CSF lactate concentrations.

We hypothesize that the integrity of the blood-brain barrier is associated with disease severity. Drug penetrates into the CSF more readily in those with the most severe disease, but the additional bactericidal activity is insufficient to prevent death and/or disability. Increased CSF drug penetration in those patients with severe TBM may also have been enhanced by higher plasma fluoroquinolone exposures. As with CSF exposure, significant U-shaped relationships between plasma fluoroquinolone exposures and poor outcomes were observed. Patients with the highest plasma exposures were older and had lower creatinine clearance rates than did those with low or intermediate exposures. Since the fluoroquinolones studied are excreted predominantly by the kidneys, impaired renal function results in increased plasma exposures (37).

As evidenced by the results of multivariable analyses, the significance of univariable U-shaped exposure-response relationships was consistently preserved when evaluated in the presence of other significant independent variables. This was evident even in the presence of the GCS, which was most strongly and consistently associated with poor outcomes. Such findings provide the opportunity to identify TBM patients at the most risk and most likely to benefit from intervention including fluoroquinolone therapy.

In summary, these findings have three important implications. First, TBM patients most likely to benefit from fluoroquinolone therapy were identified. Second, fluoroquinolone exposure-response relationships associated with improved outcomes were characterized, thus providing a paradigm for fluoroquinolone clinical development, including dose regimen selection, for the treatment of TBM patients. Third, the importance of diagnosing and treating TBM early, before the onset of coma, is highlighted.

REFERENCES

1. **Alffenaar, J. W., et al.** 2008. Plasma and cerebrospinal fluid pharmacokinetics of moxifloxacin in a patient with tuberculous meningitis. Antimicrob. Agents Chemother. **52:**2293–2295.
2. **Alffenaar, J. W., et al.** 2009. Pharmacokinetics of moxifloxacin in cerebrospinal fluid and plasma in patients with tuberculous meningitis. Clin. Infect. Dis. **49:**1080–1082.
3. **Barriere, S. L., G. W. Kaatz, and S. M. Seo.** 1989. Enhanced elimination of ciprofloxacin after multiple-dose administration of rifampin to rabbits. Antimicrob. Agents Chemother. **33:**589–590.
4. **Barsić, B., et al.** 1991. Entry of ciprofloxacin into cerebrospinal fluid during bacterial, viral and tuberculous meningitis. Neurol. Croat. **40:**111–116.
5. **Bayer HealthCare Pharmaceuticals, Inc.** 2008. Cipro (ciprofloxacin) tablets and suspension package insert. Bayer HealthCare Pharmaceuticals, Inc., Wayne, NJ.
6. **Beal, S., L. B. Sheiner, A. Boeckmann, and R. J. Bauer.** 2009. NONMEM user's guides (1989–2009). Icon Development Solutions, Ellicott City, MD.
7. **Berning, S. E., T. A. Cherry, and M. D. Iseman.** 2001. Novel treatment of meningitis caused by multidrug-resistant *Mycobacterium tuberculosis* with intrathecal levofloxacin and amikacin: case report. Clin. Infect. Dis. **32:**643–646.
8. **Bristol-Myers Squibb Company.** 2002. Tequin (gatifloxacin) tablets and injection package insert. Bristol-Myers Squibb Company, Princeton, NJ.
9. **British Medical Research Council.** 1948. Streptomycin treatment of tuberculous meningitis. Lancet **i:**582–596.
10. **Chan, E. D., and M. D. Iseman.** 2008. Multidrug-resistant and extensively drug-resistant tuberculosis: a review. Curr. Opin. Infect. Dis. **21:**587–595.
11. **Conde, M. B., et al.** 2009. Moxifloxacin versus ethambutol in the initial treatment of tuberculosis: a double-blind, randomized, controlled phase II trial. Lancet **373:**1183–1189.
12. **de Lange, E. C.** 2004. Potential role of ABC transporters as a detoxification system at the blood-CSF barrier. Adv. Drug Deliv. Rev. **56:**1793–1809.
13. **DeVincenzo, J. P., S. E. Berning, C. A. Peloquin, and R. N. Husson.** 1999. Multidrug-resistant tuberculosis meningitis: clinical problems and concentrations of second-line antituberculous medications. Ann. Pharmacother. **33:**1184–1188.
14. **Disratthakit, A., and N. Doi.** 2010. *In vitro* activities of DC-159a, a novel fluoroquinolone, against *Mycobacterium* species. Antimicrob. Agents Chemother. **54:**2684–2686.
15. **Forrest, A., C. H. Ballow, D. E. Nix, M. C. Birmingham, and J. J. Schentag.** 1993. Development of a population pharmacokinetic model and optimal sampling strategies for intravenous ciprofloxacin. Antimicrob. Agents Chemother. **37:**1065–1072.
16. **Fung-Tomc, J., et al.** 2000. *In vitro* antibacterial spectrum of a new broad-spectrum 8-methoxy fluoroquinolone, gatifloxacin. J. Antimicrob. Chemother. **45:**437–446.
17. **Gumbo, T., A. Louie, M. R. Deziel, and G. L. Drusano.** 2005. Pharmacodynamic evidence that ciprofloxacin failure against tuberculosis is not due to poor microbial kill but to rapid emergence of resistance. Antimicrob. Agents Chemother. **49:**3178–3181.
18. **Hosmer, D. W., and S. Lemeshow.** 1989. Applied logistic regression. Wiley, New York, NY.
19. **Hosoglu, S., et al.** 2002. Predictors of outcome in patients with tuberculous meningitis. Int. J. Tuberc. Lung Dis. **6:**64–70.
20. **Maeda, T., et al.** 2007. Identification of influx transporter for the quinolone antibacterial agent levofloxacin. Mol. Pharm. **4:**85–94.
21. **Ogawa, M., H. Suzuki, Y. Sawada, M. Hanano, and Y. Sugiyama.** 1994. Kinetics of active efflux via choroid plexus of beta-lactam antibiotics from the CSF into the circulation. Am. J. Physiol. **266:**R392–R399.
22. **Ooie, T., H. Suzuki, T. Terasaki, and Y. Sugiyama.** 1996. Characterization of

the transport properties of a quinolone antibiotic, fleroxacin, in rat choroid plexus. Pharm. Res. **13:**523–527.

23. **Ortho-McNeil-Janssen Pharmaceuticals, Inc.** 2009. Levaquin (levofloxacin) tablets and injection package insert. Ortho-McNeil-Janssen Pharmaceuticals, Inc., Raritan, NJ.

24. **Peloquin, C. A., et al.** 2008. Population pharmacokinetics of levofloxacin, gatifloxacin, and moxifloxacin in adults with pulmonary tuberculosis. Antimicrob. Agents Chemother. **52:**852–857.

25. **Preston, S. L., et al.** 1998. Levofloxacin population pharmacokinetics and creation of a demographic model for prediction of individual drug clearance in patients with serious community-acquired infection. Antimicrob. Agents Chemother. **42:**1098–1104.

26. **Rathore, M. S., and D. K. Majumdar.** 2006. Effect of formulation factors on *in vitro* transcorneal permeation of gatifloxacin from aqueous drops. AAPS PharmSciTech **7:**57.

27. **Rodriguez, J. C., M. Ruiz, A. Climent, and G. Royo.** 2001. *In vitro* activity of four fluoroquinolones against *Mycobacterium tuberculosis*. Int. J. Antimicrob. Agents **17:**229–231.

28. **Rodriguez, J. C., M. Ruiz, M. Lopez, and G. Royo.** 2002. *In vitro* activity of moxifloxacin, levofloxacin, gatifloxacin and linezolid against *Mycobacterium tuberculosis*. Int. J. Antimicrob. Agents **20:**464–467.

29. **Ruiz-Serrano, M. J., et al.** 2000. *In vitro* activities of six fluoroquinolones against 250 clinical isolates of *Mycobacterium tuberculosis* susceptible or resistant to first-line antituberculosis drugs. Antimicrob. Agents Chemother. **44:**2567–2568.

30. **Singh, M., et al.** 2009. *In vitro* effect of fluoroquinolones against *Mycobac-terium tuberculosis* isolates from Agra & Kanpur region of north India. Indian J. Med. Res. **129:**542–547.

31. **Teasdale, G., and B. Jennett.** 1974. Assessment of coma and impaired consciousness. A practical scale. Lancet **ii:**81–84.

32. **Thwaites, G. E., et al.** 2003. Pathophysiology and prognosis in Vietnamese adults with tuberculosis meningitis. J. Infect. Dis. **188:**1105–1115.

33. **Thwaites, G. E., et al.** 2004. Dexamethasone for the treatment of tuberculous meningitis in adolescents and adults. N. Engl. J. Med. **351:**1741–1751.

34. **Thwaites, G. E., et al.** 2005. The influence of HIV infection on clinical presentation, response to treatment, and outcome in adults with tuberculous meningitis. J. Infect. Dis. **192:**2134–2141.

35. **Thwaites, G. E., and T. H. Tran.** 2005. Tuberculous meningitis: many questions, too few answers. Lancet Neurol. **4:**160–170.

36. **Tsukamura, M., E. Nakamura, S. Yoshii, and H. Amano.** 1985. Therapeutic effect of a new antibacterial substance ofloxacin (DL8280) on pulmonary tuberculosis. Am. Rev. Respir. Dis. **131:**352–356.

37. **Turnidge, J.** 1999. Pharmacokinetics and pharmacodynamics of fluoroquinolones. Drugs **58**(Suppl. 2)**:**29–36.

38. **Upton, A., A. Woodhouse, R. Vaughan, S. Newton, and R. Ellis-Pegler.** 2009. Evolution of central nervous system multidrug-resistant *Mycobacterium tuberculosis* and late relapse of cryptic prosthetic hip joint tuberculosis: complications during treatment of disseminated isoniazid-resistant tuberculosis in an immunocompromised host. J. Clin. Microbiol. **47:**507–510.

39. **van Doorn, H. R., et al.** 2008. Fluoroquinolone resistance detection in *Mycobacterium tuberculosis* with locked nucleic acid probe real-time PCR. Int. J. Tuberc. Lung Dis. **12:**736–742.